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STUDIES ON THE FINE STRUCTURE OF THE TERMINAL BRANCHES OF THE BILIARY TREE

I. THE MORPHOLOGY OF NORMAL BILE CANALICULI, BILE PRE-DUCTULES (DUCTS OF HERING) AND BILE DUCTULES

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Numerous papers have dealt in some detail with the fine structure of bile canaliculi and their relation to parenchymal liver cells and to the spaces of Disse.[1-10] Yamada[11] analyzed the fine structure of the gallbladder of mice. Only one paper referred to the electron microscopic appearance of bile ductules in portal tracts.[12]

The purpose of this study is to delineate the morphology of the ultimate and penultimate branches of the biliary tree as a base line for investigations of pathologic alterations of these structures. Observations of other investigators will be examined in the light of new information gained by the use of metallic impregnation and staining techniques applied to electron microscopy.

MATERIAL AND METHODS

Normal livers of dogs, rabbits and rats, in addition to human surgical biopsy specimens, were examined. Small fragments of tissue were fixed in Palade's buffered osmium tetroxide (pH 7.4) for 2 hours and processed through graded alcohols in the usual manner. A few blocks of tissue were transferred, after a normal fixation period in Palade's osmium tetroxide, to the first solution of alcohol containing 5 per cent uranyl acetate for one-half hour before further dehydration. All tissues were finally embedded in butyl-methyl methacrylate (8:1). Ultrathin sections were then cut on a Porter-Blum ultramicrotome and examined unstained with a phase contrast microscope. Poorly preserved tissues were rejected at this stage, attention being paid particularly to the liver plate-portal connective tissue junction. This tended to become disrupted, probably as a result of uneven polymerization of methacrylate.

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Some sections were picked up on Formvar-coated grids without further processing. These included unstained sections of osmium tetroxide-fixed material as well as those from uranyl acetate-impregnated blocks. Other ultrathin sections were floated on and stained in the following manner: (a) with silver methenamine, with and without prior periodic acid oxidation (PASM and SM) [13]; (b) with phosphotungstic acid (10 per cent solution) for 15 minutes, 1 hour and 18 hours respectively (PTA). This is basically an adaptation of the PTA stain for bile canaliculi suggested by Mallory [14]; (c) with lead hydroxide by the method of Dalton and Zeigel [15]; (d) with protargol (silver proteinate) by the method of Movat.[16]

The sections were examined in an RCA EMU-3-E microscope at magnifications ranging from 1,400 to 32,000. Further enlargement was obtained by means of a Leitz Focomat II enlarger.

Terminology

The nomenclature of the terminal branches of the biliary tree is somewhat confused. The term bile canaliculus [17–20] is used synonymously with bile capillary.[20,21] Others reserve the latter term for connecting channels between the lobular and portal tract conducting systems.[22] Many synonyms are being used to designate these connecting canals such as canal of Hering,[19] duct of Hering [18] and *"Zwischenstücke."* [23] The latter is translated by Popper and Schaffner [19] as "intermediary portion" and by Schiff [21] as "intermediate canal." In addition, Eppinger [24] used the term ampulla to describe the dilated junction of bile canaliculi and bile ductules. This term is also used by Schiff.[21] However, Popper and Schaffner [19] point out that the term ampulla may be used to designate the entire intermediary portion.

Bile ductules and bile ducts are the designations used by most authors for the remaining larger branches of the biliary tree in portal tracts. Some use the term terminal bile ducts or cholangioles for the smallest branches in this system.[20] Others lump these entities together under the term of bile ducts (*Gallengänge*),[22] axial branches of bile ducts [21] or interlobular bile ducts.[18,19,21]

We consider that the terms canaliculus or canal refer to a passage which possesses no specialized lining of its own, i.e., it is merely a tissue space. On the other hand, the term ductule or duct implies a channel provided with its own specialized lining cells. The term bile canaliculus is acceptable for the intralobular portion of the system since it is formed merely by a gap between parenchymal liver cells. It will be shown that in the interlobular portion of the system all passages are lined by biliary epithelial cells even though in some parts they are formed by a mere focal separation of the limiting membranes of these cells.

It is proposed to employ the following terminology for the terminal branches of the biliary tree:

(1) *Bile canaliculi* will be the term used for intralobular passages bounded by parenchymal liver cells.

(2) *Bile pre-ductules* will be employed in the designation of the connecting channels between bile canaliculi and bile ductules in portal tracts. (The term duct of Hering will be used synonymously with this.)

(3) *Bile ductules and bile ducts* will be applied to the larger branches of the biliary tree in extralobular locations.

The ampullary portion of this system could not be identified in electron micrographs, and the term will therefore not be used.

Results

The fine structure of parenchymal liver cells has been adequately reviewed in the literature. Biliary epithelium, on the other hand, has not been examined in detail. Because of its importance in relation to the course of the terminal branches of the biliary tree, its appearance will be analyzed before the course of the ultimate and penultimate pathways of bile conduction will be outlined.

The Fine Structure of Biliary Epithelium

These cells in biliary passages have been described as cuboidal, with various amounts of fairly homogeneous cytoplasm with fewer mitochondria and less ergastoplasm than parenchymal liver cells.[12] The microvilli of these cells have been said to be shorter and farther apart than in liver cells.[27] The over-all shape of the cells is usually pyramidal with a slight apical narrowing at the lumen surface (Figs. 7 and 10). Occasional cells have elongated processes projecting toward the lumen from a wide base so that their participation in the formation of the lumen boundary is narrow when observed in a two-dimensional plane.

The lateral cell walls form closely interlocking cytoplasmic processes (Figs. 7, 10 and 18). The cell membranes in human tissue each measure 460 Å in thickness and are separated from each other by a paler intercellular zone measuring on an average of 1,860 Å in width. This compares with the measurements in the gallbladder of mice where the cell membranes measure 150 Å and the intervening space 100 Å.[11] In the human subject the complexity of the interlocking of cells is far greater than in the various animal species examined. In general, the plications are more numerous toward the base of the cell pyramid. Desmosomal cytoplasmic densities are noted occasionally around the cell membranes in the immediate vicinity of the lumen.

The lumen surface of biliary epithelium as well as focal gaps between lateral cell walls are provided with microvilli (Figs. 7 to 13, 16, 17 and 19). These are progressively larger with increasing width of the lumen. The microvilli are provided with a double-membrane covering (outer layers 626 Å each and inner clear zone 660 Å). This is rather difficult to

demonstrate because of the frequent tangential direction of sections through the surface of the microvilli (Fig. 19).

Contrary to the statement of others,[12] mitochondria are frequently abundant in biliary epithelium (Fig. 7) though variations are noted in their number and distribution. In agreement with others,[18,19] mitochondria are frequently found to be layered or aggregated in two zones, one adjacent to the lumen of ducts and ductules and another adjacent to the outer border of these cells. The over-all shape of the mitochondrial corpuscles varies from round to elongated. The cristae mitochondriales usually traverse almost the entire width of the corpuscle, dividing the matrix into multiple slit-like intercommunicating compartments (Fig. 15). This is akin to the structure of the mitochondria in cells of mesenchymal orgin. It is distinct from that of parenchymal liver cell mitochondria in which the cristae generally project only about one third of the distance from the periphery towards a hypothetical center in a spoke-like fashion, leaving a considerable amount of free matrix in the lumen (Fig. 14). The "opaque bodies" which are prominent in parenchymal cell mitochondria (Fig. 14) are less numerous and distinct in biliary epithelium.

The ergastoplasm (endoplasmic reticulum) is mainly of the smooth-surfaced, agranular kind. In lead hydroxide-stained sections, only a few ergastoplasmic membranes are provided with microsomal granules. Free cytoplasmic ribosomes were equally scarce. Golgi areas are usually prominent and located on the lumen side of the nucleus (Fig. 7). Centrosomes are seen only very rarely.

The nuclei are situated in the basal half of the cells. The karyoplasm is evenly distributed, and, unlike parenchymal cells, peripheral condensation of this substance and nucleoli are rarely prominent. Electron-dense microbodies are found within the cytoplasm frequently (Figs. 12, 13, and 16 to 18). These are usually membrane-enclosed. Their main component is probably lipid, but the possibility that at least some also contain carbohydrate is supported by their marked staining intensity with PASM[13] (Figs. 8, 10 and 11).

In addition to the characteristic cells, others are found in the penultimate branches of the biliary tree, located near the outer margin of the passages. In two-dimensional sections these do not reach the lumen. They were designated as intercalated cells (Fig. 7) and are generally characterized by less complex infoldings of cell membranes and by a lesser number of cytoplasmic organelles. These cells must be distinguished from mesenchymal elements which are often found intermingled with biliary epithelial cells in the vicinity of the basement membrane of bile ductules and ducts, particularly in rabbits. Such a distinction is occasionally difficult to make.

The basement membrane on which the outer surface of biliary epithelium rests is usually a simple structure 3,100 Å in width, with a space of approximately 2,780 Å separating it from the limiting membrane of the cells (Fig. 16). In human tissue the basement membrane is often a more complex, branching structure with both its thickness and its distance from biliary epithelium extremely variable (Figs. 7, 8 and 11). The membrane stands out clearly with the PTA stain (Fig. 16). Its extreme PASM positivity (Figs. 8 and 11) is probably related to its glycoprotein composition.[13] It is not clear whether the membrane is elaborated by the cells or by fibroblasts in the connective tissue beyond the basement membrane.

The Course of the Terminal Pathways of Bile Conduction

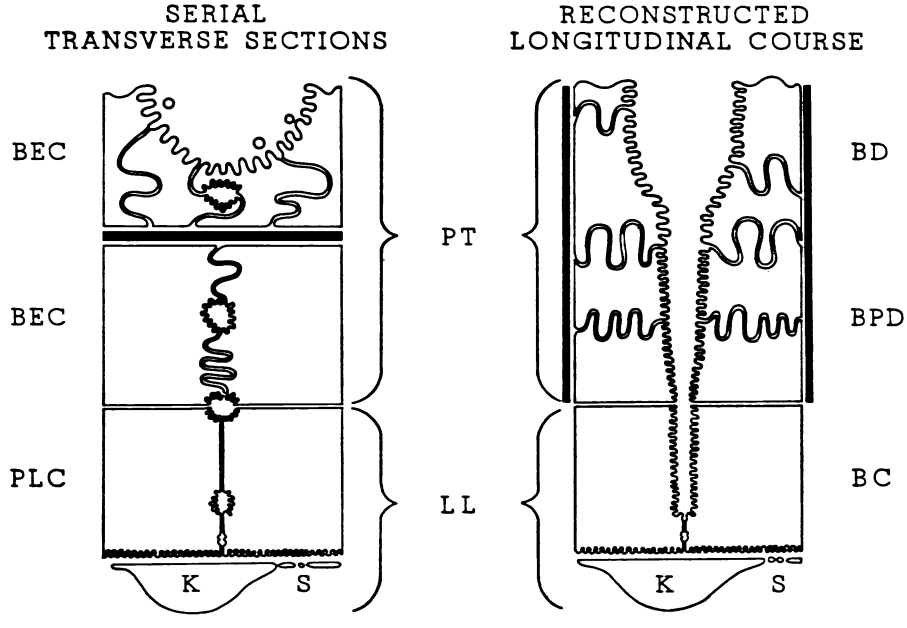
The intralobular portion of the biliary tree—the bile canaliculi—are merely gaps between adjacent parenchymal liver cells (Figs. 1 to 6). The bile pre-ductules (ducts of Hering) are similarly located between two adjacent biliary epithelial cells in portal tracts (Figs. 9 and 17). Bile ductules in extralobular areas are formed by rosettes of biliary epithelial cells surrounding a lumen (Figs. 7, 8, 10, 12, 16 and 17). Because of extremely tortuous courses, these passages are seen mostly in cross section. Text-figure 1 shows a series of such cross sections on the left side and on the right a surmised reconstruction of the longitudinal course of these channels.

A thorough search of the periphery of lobules revealed infrequent points of contact between parenchymal liver cells and biliary epithelium. This is observed to occur in one of 3 ways: (a) a side-to-side contact of the cell membranes of these cells; (b) an interposition of one or several biliary epithelial cells between two parenchymal cells of the peripheral liver plate (Fig. 10); (c) a herniation of a parenchymal liver cell in the periphery of a lobule into a basement membrane-bounded canal lined by biliary epithelium (Fig. 11).

A basement membrane (thick black line in Text-fig. 1) is never interposed between a biliary epithelial cell and parenchymal cell at their point of contact (Figs. 10 and 11). Higher up the tree, in ductules and ducts, the rosettes of biliary epithelium lining them are completely surrounded by a basement membrane (Figs. 8 and 16). Thus in cross section it appears that the duct of Hering would have to traverse the basement membrane in order to reach the lumen of the ductules (Text-fig. 1, left). A longitudinal reconstruction (Text-fig. 1, right), however, shows that the biliary pathway is a continuous channel which never needs to cross this membrane.

Both bile canaliculi and ducts of Hering form a complex network of channels which are probably branching. It is at present not clear whether

each duct of Hering corresponds to one canaliculus or whether canaliculi form a confluence prior to entering into the confines of biliary epithelial cells where the channels again branch. Only serial sections of these areas



TEXT-FIGURE 1. Outline of the course of the terminal branches of the biliary tree.

of transition from canaliculi to pre-ductules will supply the answer to this problem.

The Fine Structure of Bile Canaliculi

Bile canaliculi are bounded only by the limiting membranes of adjacent liver cells.[8] With PTA stain, an intensification of the staining properties of all such membranes is noted where they form the wall of biliary passages. By these means, all microvilli are found to have a double lining membrane. This is most difficult to resolve at a canalicular level since the separation of the two outer layers seems to be less than 12 Å. Higher up the biliary tree this is seen more easily (Fig. 19). The over-all topography of these channels has been adequately reviewed.[1-10] As most observers have noted, connections between bile canaliculi and spaces of Disse cannot be demonstrated (Fig. 6). This observation contradicts the findings of Rouiller.[3,4,25] The differentiation between

sinusoidal recesses and canals and bile canaliculi is aided by the use of the PTA stain since reticulin fibers can easily be observed in the former [7] and their absence noted in the latter.

The number of bile canaliculi in a given low-power field (× 1,400) of normal hepatic parenchyma is extremely variable, and alleged reductions in numbers must perforce constitute merely an impression. The width of canalicular lumens has been stated to be one μ and the lumen round.[6] Even in well-preserved tissue, variations are noted in both width and shape of the lumen, depending on the angle of sectioning (Fig. 3). Occasionally a section passes through in such a way that the impression is gained that no lumen is present. In other instances channels somewhat wider than one μ are noted (Fig. 5). Tangential sections probably do not account for all such variations, and the functional status of neighboring liver cells may have a bearing on this.[25] Interpretation of pathologic alterations can therefore be based only on striking deviations from normal.

The fine structure of the microvilli of liver cells, where these project into bile canaliculi, has been described by others.[6] They are said to be regularly distributed and equal in width (0.05 to 0.1 μ). Observations in this laboratory show variations in the numbers, length and width of the microvilli in normal tissues (Figs. 4 and 5), confirming the views expressed by others.[25] Judgment of pathologic alterations of microvillous borders must therefore be based in the final analysis on impressions of grossly disturbed patterns of these structures rather than on exact measurements.

The contents of bile canaliculi can be visualized only on rare occasions in normal tissues as a faintly electron-opaque, apparently nonparticulate, cloudy material (Fig. 2). In PASM-stained sections diastase-digestible glycogen granules are found occasionally in the lumens as well as in the microvilli.[13]

It has been suggested that rat liver cell mitochondria are oriented in sinusoid-canalicular directions as part of the over-all polarization of cytoplasmic organelles, and that the Golgi apparatus, which is known to be related to excretory function,[26] is always [2] or commonly [17] situated adjacent to bile canaliculi.[2] Our observations often showed random orientation of mitochondria and absence of Golgi material from the neighborhood of canaliculi. The latter finding may be related to variable levels of sectioning. Polarization of parenchymal cell mitochondria and of the endoplasmic reticulum in a canalicular direction is seen very rarely (Fig. 1). Small, probably pinocytic vesicles are usually abundant in the cell cytoplasm in the immediate neighborhood of canaliculi (Figs. 1 and 2),

where the cytoplasmic matrix is frequently more highly electron-dense than in the remainder of the cell (Fig. 2).

The Fine Structure of Bile Pre-ductules and Ductules. At the point of origin, the ducts of Hering are always bounded on one side by one or more parenchymal liver cells, and on the other by one or several biliary epithelial cells. From then onward they pass between two biliary epithelial cells and are seen in cross-section as mere gaps between the cell membranes which are provided with microvilli on these surfaces (Figs. 9 and 17). The ducts of Hering end at the point of confluence with bile ductules. Bile ductules are channels bounded by a rosette of biliary epithelial cells which are provided on their entire lumen surface by microvilli (Figs. 8 to 10, 12, 13, 16 and 17).

The distinction between pre-ductules and ductules in cross section is easily apparent (Text-fig. 1, left). In longitudinal section, however, the distinction is impossible to make since the difference depends on an assessment of the width of the lumen (Text-fig. 1, right; Fig. 8).

The length of bile pre-ductules is also difficult to assess because of their extremely tortuous course, but occasionally they appear to traverse merely the length of one biliary epithelial cell before reaching the lumen of a ductule (Figs. 10 and 11).

The width of the lumens and the size, shape and distribution of the microvilli of biliary epithelium where these project into bile pre-ductules and ductules are subject to the same variations which have been described in bile canaliculi (Figs. 7, 10, 12 and 13). It is therefore equally important in these locations to limit allegations of pathologic changes to observations of grossly disturbed normal patterns. The biliary epithelial cells which form bile pre-ductules and the outer surface of the rosettes of these cells which form bile ductules are always covered by a basement membrane. The only gap in this envelope occurs at the point of contact between biliary epithelium and parenchymal liver cells in the marginal areas of the lobules.

Discussion

The study of the fine structure of normal bile canaliculi pre-ductules and ductules shows that variations in the over-all distribution, size and shape, as well as in the size, shape and distribution of their microvilli occur and need be taken into consideration when assessing pathologic alterations of these structures. It is suggested that only major deviations from the normal pattern should be interpreted as pathologic and that little reliance should be placed within wide limits on actual measurements.

The observation that microvilli projecting into biliary passages from

both liver cells and biliary epithelium are provided with a double lining membrane needs to be interpreted with caution at present. The finding is most easily confirmed by examination of ultrathin sections stained with PTA. This method has been suggested by Mallory [14] for the staining of bile canaliculi for light microscopy and adapted by us for electron microscopy. The results show that the lining of microvilli stains extremely intensely by this method (as do collagen and reticulin fibrils and some lipid inclusions) whereas other parts of both parenchymal and biliary epithelial cells stain faintly. The lateral limiting membrane of biliary epithelium is not accentuated by this technique of staining, nor are the microvilli of liver cells which project into the perisinusoidal space of Disse.[7] Thus it appears that the accentuation may be due to a specific affinity of PTA for some material contained in or being excreted or reabsorbed through the cell membranes lining biliary passages. It is possible that the lining does not represent a true double membrane but rather a condensation of metabolites in process of transfer across the cell membrane in either direction. This possibility must be considered since the lateral cell membranes prior to forming microvilli appear to be single and then "split" upon entering the surface of these structures. It is nevertheless possible that with our available electron microscope, indentification of double membranes on lateral cell walls could not be achieved, owing to the limitations of resolution.

The complex plications of the lateral walls of biliary epithelial cells are thought to serve the purpose of permitting easy expansion of the lumen in response to increases of intraluminal pressure. It will be shown [28] that intracellular edema is also associated with a straightening of the cell boundaries. These plications appear to be common to all cells of the biliary tree since they were also noted between the lining cells of the mouse gallbladder.[11] Two points are of interest: (1) Yamada [11] noted in the gallbladder that some plications of epithelial cells did not fit into corresponding recesses of neighboring cells. This was never the case in the cells lining the biliary passages. (2) The plications were, on the whole, considerably more numerous in human than in animal tissues.

Little is known about the function of biliary epithelium at the level of bile pre-ductules and ductules. Observations reported in this study suggest that these cells are highly active metabolically, as evidenced by the large numbers of mitochondria and by their well-developed Golgi apparatus. The presence of lipid and other electron-opaque bodies in these cells suggests that such materials are being handled actively by them. Further, the presence of microvilli on the lumen surfaces of both bile pre-ductules and ductules suggests that biliary epithelium is endowed with active secretory or resorptive functions.

Summary

The fine structure of the ultimate and penultimate branches of the biliary tree was examined in normal dogs, rats, rabbits and in human liver biopsy tissue. The course of biliary pathways was traced from bile canaliculi of the lobules through bile pre-ductules (canals of Hering) in portal tracts to bile ductules. Canaliculi were shown to be formed by a focal separation of the limiting membranes of parenchymal liver cells, and pre-ductules in a similar fashion by biliary epithelial cells. Ductules were defined as the first passages which are lined by biliary epithelium in a rosette-like formation where entire cell walls form the lumen boundary. This study is intended to form the base line for investigations of the fine structure of pathologically altered pathways of bile conduction.

References

1. Ashworth, C. T., and Sanders, E. Anatomic pathway of bile formation. *Am. J. Path.*, 1960, **37**, 343–355.
2. Novikoff, A. B., and Essner, E. The liver cell; some new approaches to its study. *Am. J. Med.*, 1960, **29**, 102–131.
3. Rouiller, C. Les canalicules biliaires; étude au microscope électronique. *Compt. rend Soc. biol.*, 1954, **148**, 2008–2011.
4. Rouiller, C. Les canalicules biliaires; étude au microscope électronique. *Acta anat.*, 1956, **26**, 94–109.
5. Hampton, J. C. An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct. *Acta anat.*, 1958, **32**, 262–291.
6. Schaffner, F., and Popper, H. Morphologic studies of cholestasis. *Gastroenterology*, 1959, **37**, 565–573.
7. Steiner, J. W. Studies of allergic liver injury. I. Light, fluorescent and electron microscopic study of the effects of soluble antigen-antibody complexes. *Am. J. Path.*, 1961, **38**, 411–436.
8. Fawcett, D. W. Observations on the cytology and electron microscopy of hepatic cells. *J. Nat. Cancer Inst.*, 1955, **15**, Suppl., 1475–1503.
9. Lanzavecchia, G.; Polli, E.; Jean, G.; LeCoultre, A., and Pisani, E. Struttura della cellula epatica umana, osservata al microscopio eletronico. 1. Fegato umano normale. *Arch. ital. anat. embriol.*, 1959, **64**, 404–429.
10. Brown, D. B.; Delor, C. J.; Greider, M., and Frajola, W. J. The electron microscopy of human liver. *Gastroenterology*, 1957, **32**, 103–118.
11. Yamada, E. The fine structure of the gall bladder epithelium of the mouse. *J. Biophys. & Biochem. Cytol.*, 1955, **1**, 445–458.
12. Popper, H.; Schaffner, F.; Hutterer, F.; Paronetto, F., and Barka, T. Parenchymal fibrogenesis: the liver. *Ann. New York Acad. Sc.*, 1960, **86**, 1075–1088.
13. Steiner, J. W., and Lambert, B. F. Unpublished observations.
14. Mallory, F. B. Pathological Technique; a Practical Manual for Workers in Pathologic Histology. W. B. Saunders Co., Philadelphia, 1938, 434 pp.
15. Dalton, A. J., and Zeigel, R. F. A simplified method of staining thin sec-

tions of biological material with lead hydroxide for electron microscopy. *J. Biophys. & Biochem. Cytol.*, 1960, 7, 409–410.

16. MOVAT, H. Z. Unpublished observations.
17. HAM, A. W. Histology. J. B. Lippincott Co., Philadelphia and London, 1957, ed. 3, 894 pp.
18. MAXIMOW, A. A., and BLOOM, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia and London, 1957, ed. 7, 628 pp.
19. POPPER, H., and SCHAFFNER, F. Liver: Structure and Function. The Blakiston Div., McGraw-Hill Book Co., Inc., New York, Toronto and London, 1957, 777 pp.
20. SHERLOCK, S. Diseases of the Liver and Biliary System. Charles C Thomas, Springfield, Ill., 1955, 720 pp.
21. SCHIFF, L. Diseases of the Liver. J. B. Lippincott Co., Philadelphia and Montreal, 1956, 738 pp.
22. LA MANNA, S. Beitrag zur Kenntnis der Beziehungen zwischen Hepatose und Gelbsucht. *Virchows Arch. path. Anat.*, 1937, **300**, 398–428.
23. CLARA, M. Untersuchungen an der menschlichen Leber. I. Über den Übergang der Gallenkapillaren in die Gallengänge. *Ztschr. mikr.-anat. Forsch.*, 1930, **20**, 584–607.
24. EPPINGER, H. Die Leberkrankheiten; allgemeine und spezielle Pathologie und Therapie der Leber. J. Springer, Vienna, 1937, 801 pp.
25. ROUILLER, C. Contribution de la microscopie électronique à l'étude du foie normal et pathologique. *Ann. anat. path.*, 1957, **2**, 548–562.
26. KIRKMAN, H., and SEVERINGHAUS, A. E. A review of the Golgi apparatus. *Anat. Rec.*, 1937–1938, **70**, 413–431.
27. SCHAFFNER, F., and POPPER, H. Electron microscopic study of human cholestasis. *Proc. Soc. Exper. Biol. & Med.*, 1957, **101**, 777–779.
28. STEINER, J. W., and CARRUTHERS, J. S. Unpublished observations.

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[*Illustrations follow*]

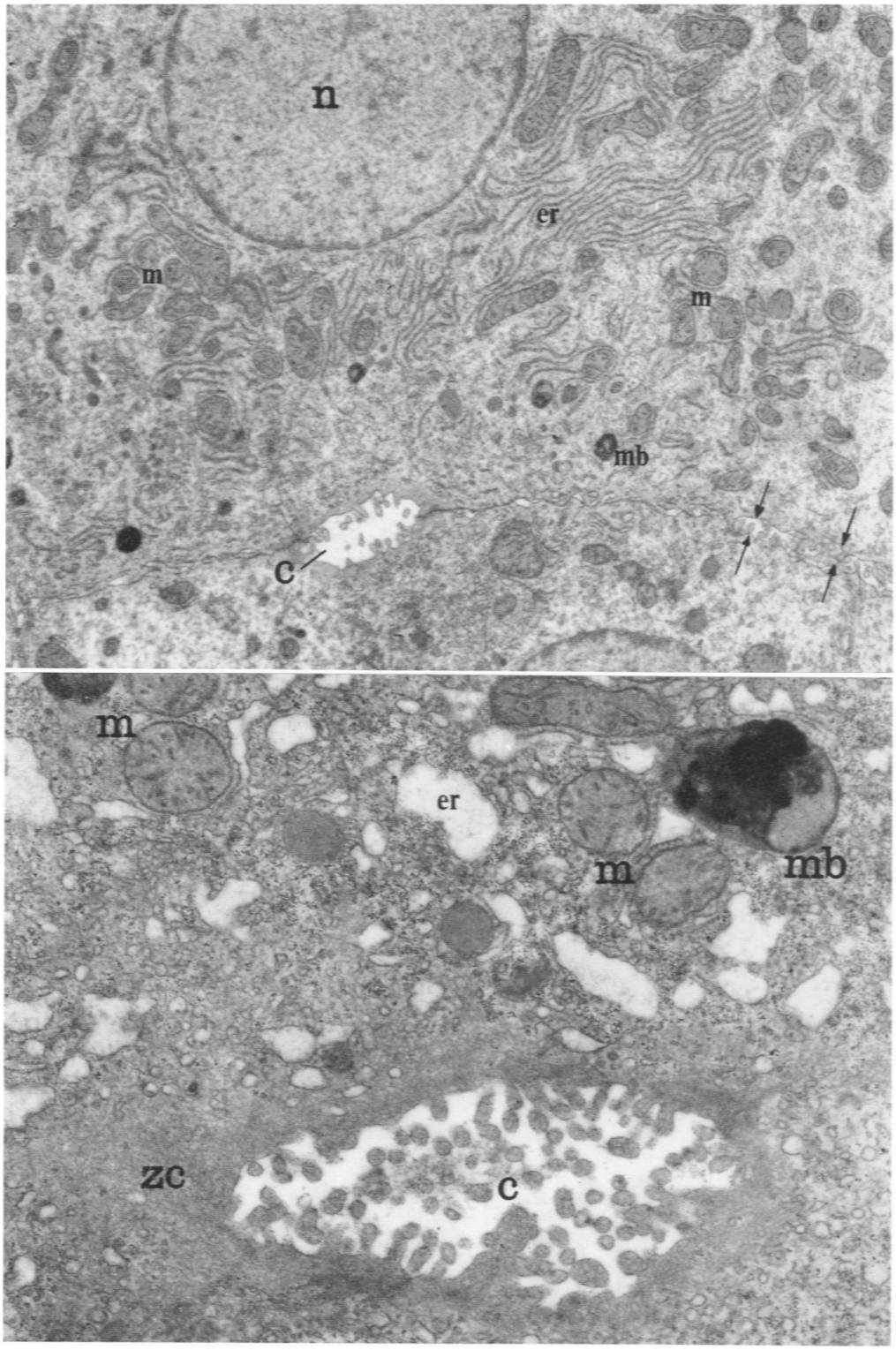
LEGENDS FOR FIGURES

Key:

bec = biliary epithelial cell
bm = basement membrane
c = bile canaliculus
clg = collagen fibrils
cm = cell membrane
d = bile ductule
db = mitochondrial dense bodies
dr = bile ductular recess
er = endoplasmic reticulum (ergastoplasm)
G = Golgi apparatus
ic = intercalated cell of bile ductule
l = lumen
m = mitochondrion
mb = microbodies (lipid, lipofuscin, "peribiliary" and glycoprotein cell inclusions)
mv = microvillus
n = nucleus
nm = nuclear membrane (envelope)
pd = bile pre-ductule (duct of Hering)
plc = parenchymal liver cell
pr = perisinusoidal recess
ps = perisinusoidal space (space of Disse)
rnp = ribose-nucleoprotein granules
s = sinusoid
t = Kupffer cell trabecula
zc = zone of cytoplasmic condensation

FIG. 1. Electron micrograph of two adjacent liver cells with a focal separation of the cell membranes forming the bile canaliculus. The cell membranes of the remaining portions of the liver cells are provided with a few microvilli (arrows) up to a short distance from the canalicular lumen. This represents the lining of extremely narrow perisinusoidal canals. Note the rarely observed polarization of mitochondria and of the endoplasmic reticulum toward the bile canaliculus. Numerous small vacuoles (possibly pinocytic vesicles) are present in the cytoplasm immediately adjacent to the canalicular wall. Protargol stain. × 8,120.

FIG. 2. A bile canaliculus is seen between liver cells. Note the presence of a faintly gray amorphous substance in the lumen and the condensation of the cytoplasmic matrix around the periphery of the canaliculus. Numerous small, smooth-surfaced vesicles are present outside this zone. Two microbodies and a lipofuscin body are present in the liver cell on the right side. The endoplasmic reticulum of this cell is slightly dilated. Protargol stain. × 25,600.



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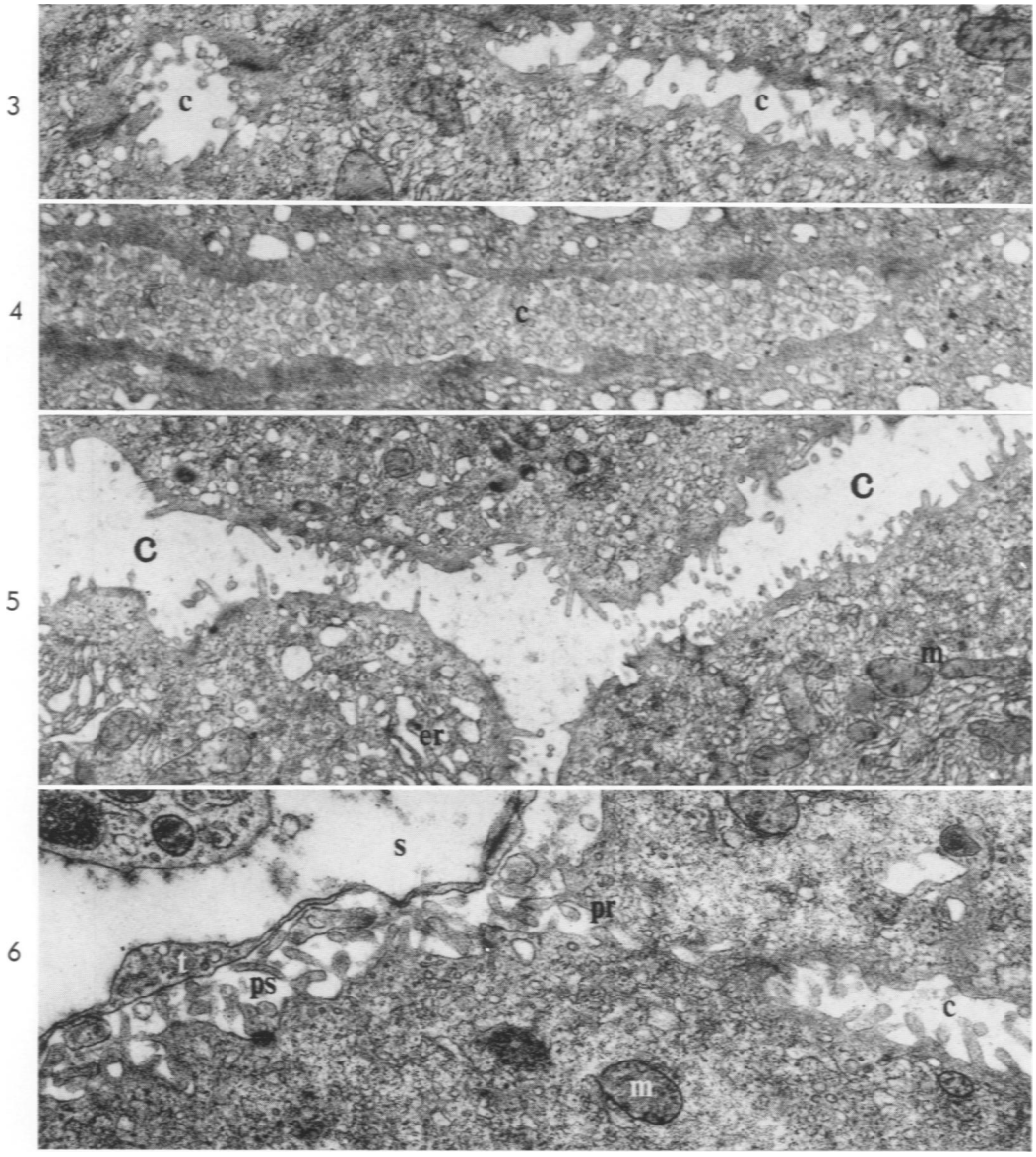


FIG. 3. The difference in appearance and size of bile canaliculi when seen in transverse (left) and longitudinal (right) section is shown. Protargol stain. × 16,000.

FIG. 4. The microvilli in this bile canaliculus are so numerous as to give the impression of occlusion. Protargol stain. × 17,920.

FIG. 5. Longitudinal section of a bile canaliculus. The angular recess in the lower center represents the confluence of two bile canaliculi. Note the marked variation in the length and distribution of the microvilli. Protargol stain. × 10,640.

FIG. 6. A sinusoid (left) is separated from the space of Disse by a terminal trabecula of a Kupffer cell. A perisinusoidal recess extends toward a bile canaliculus. Despite close approximation, note the absence of a communication between these two structures. Protargol stain. × 17,920.

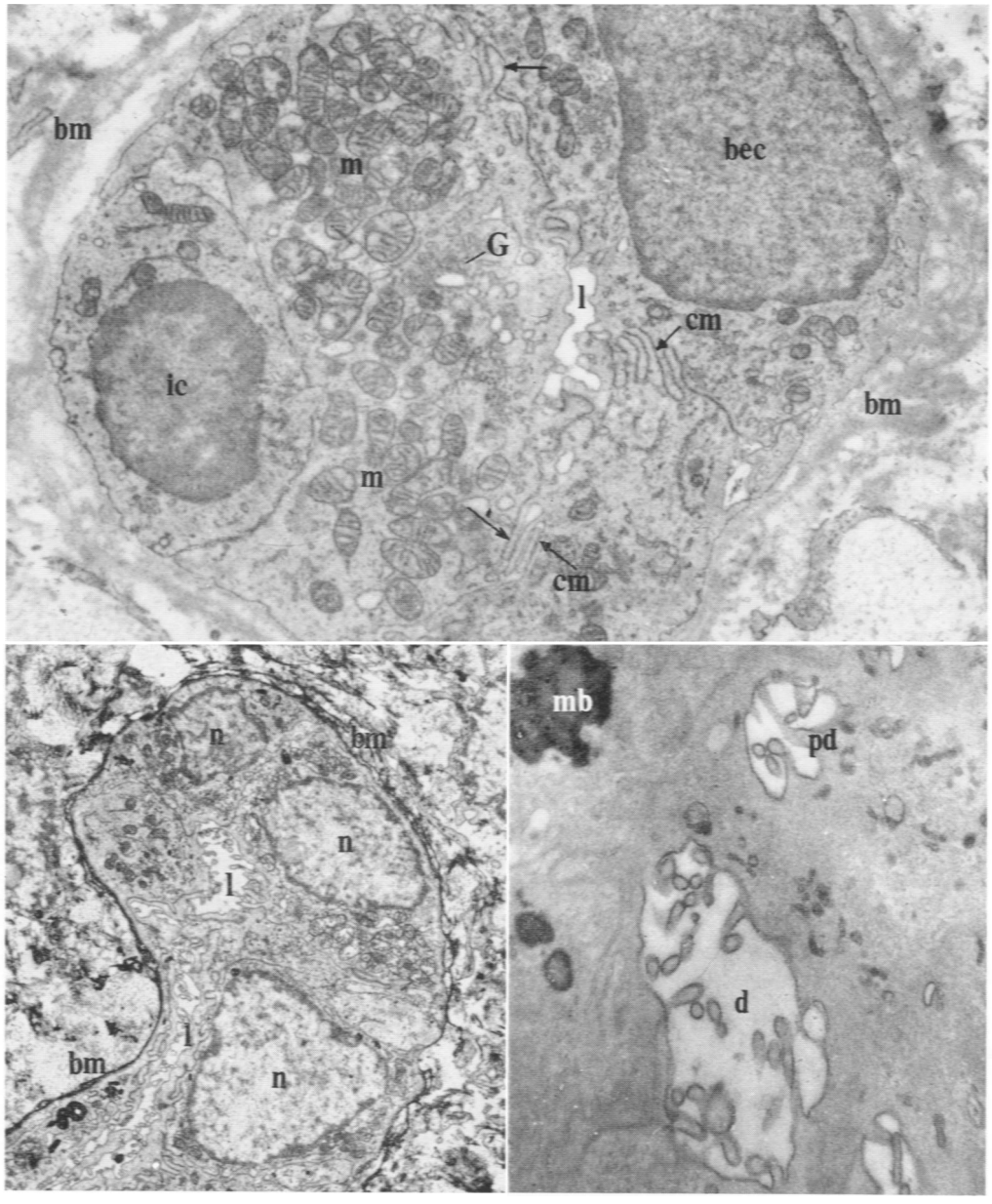


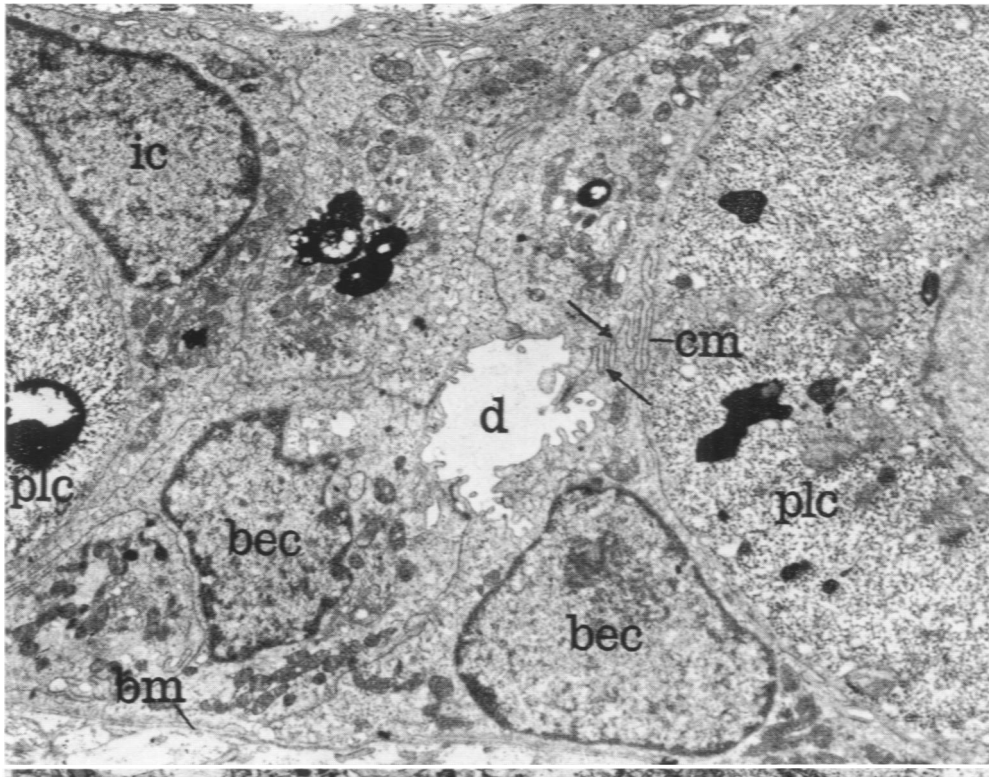
FIG. 7. Cross section of a bile ductule, the lumen of which is lined by 3 cells separated by plicated cell membranes (arrows). The intercalated cell does not reach the lumen. Note the large number of mitochondria and the prominent Golgi zone. The basement membrane is a complex branching structure. Uranyl acetate impregnation. × 12,800.

FIG. 8. An interlobular biliary pathway seen in transverse (upper center) and longitudinal (lower left corner) section. It is impossible to say whether the latter represents a bile pre-ductular or a ductular lumen. The basement membrane is a complex branching structure. Periodic acid-silver methenamine stain. × 5,040.

FIG. 9. A bile ductule and two afferent pre-ductules seen in cross section. Phosphotungstic acid stain. × 22,400.

FIG. 10. Six biliary epithelial cells are interposed between two parenchymal cells of a peripheral liver plate. The latter can be recognized partly by the presence of glycogen granules in their cytoplasm. The former show the characteristic mode of interlocking of the cell membranes (arrow). A basement membrane can be seen adjacent to the biliary epithelial cells at the lower margin of the photograph. The lumen is that of a bile ductule. The afferent duct of Hering cannot be seen at this level of sectioning. Periodic acid-silver methenamine stain. × 7,840.

FIG. 11. A parenchymal liver cell has herniated into a basement membrane-enclosed channel formed by biliary epithelium. The large mitochondria of the former distinguish it from the relatively small mitochondrial corpuscles of the latter (see Figs. 14 and 15). Note the narrow lumen of the bile ductule and the plications of the cell membranes (arrow). Periodic acid-silver methenamine stain. × 5,740.



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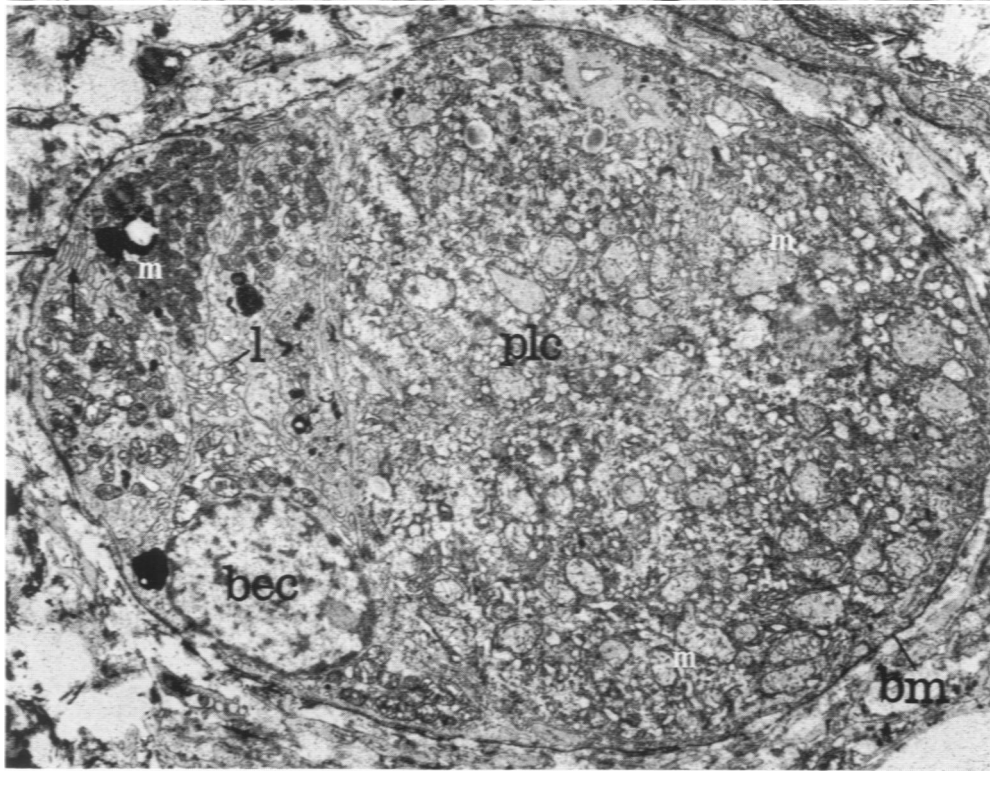

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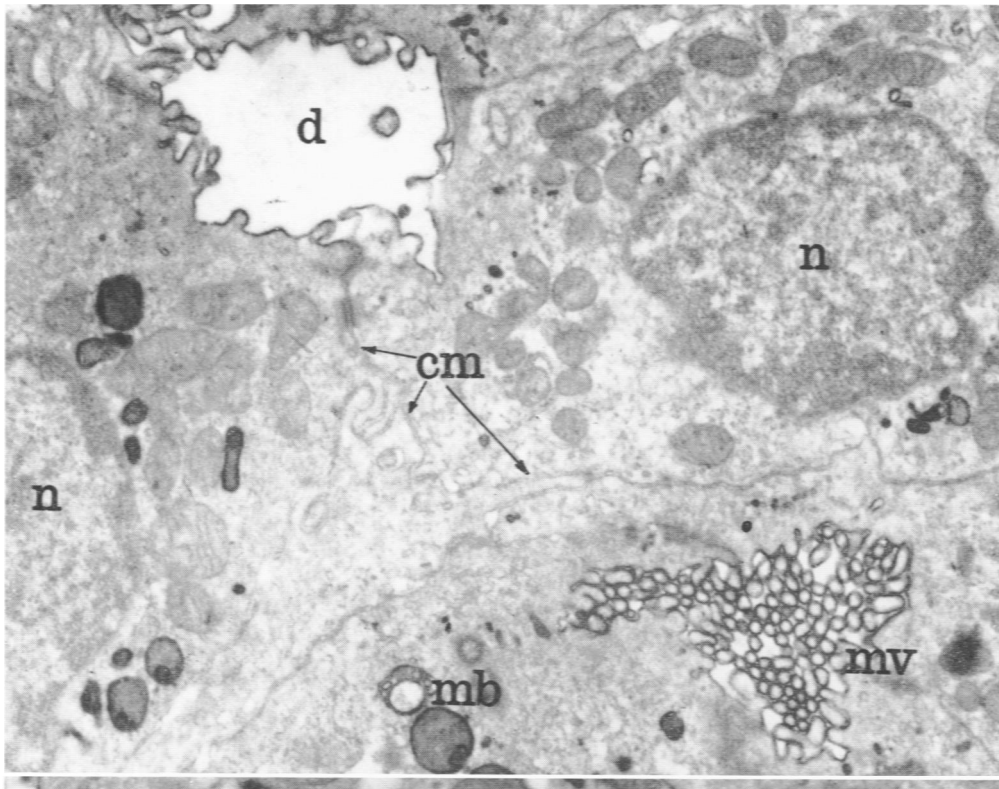
FIG. 12. Cross section of two bile ductular lumens can be seen. The one in the left upper corner of the photograph shows a widely patent lumen whereas the one in the right lower corner shows the lumen almost totally occluded by a mass of microvilli. The latter appearance is thought to be the result of a section through the periphery of a lumen. Note the small lipid inclusions scattered throughout the cytoplasm of the biliary epithelial cells. Phosphotungstic acid stain. × 19.040.

FIG. 13. Longitudinal section of a bile ductule, showing a marked variation in the size, shape and distribution of the microvilli. A second, smaller channel in the left lower center of the illustration is separate from the cell membranes which skirt it on its right. It is therefore interpreted as a recess of the main channel rather than a pre-ductule. Note the intense staining of the cell membrane on the lumen side. Phosphotungstic acid stain. × 17,600.



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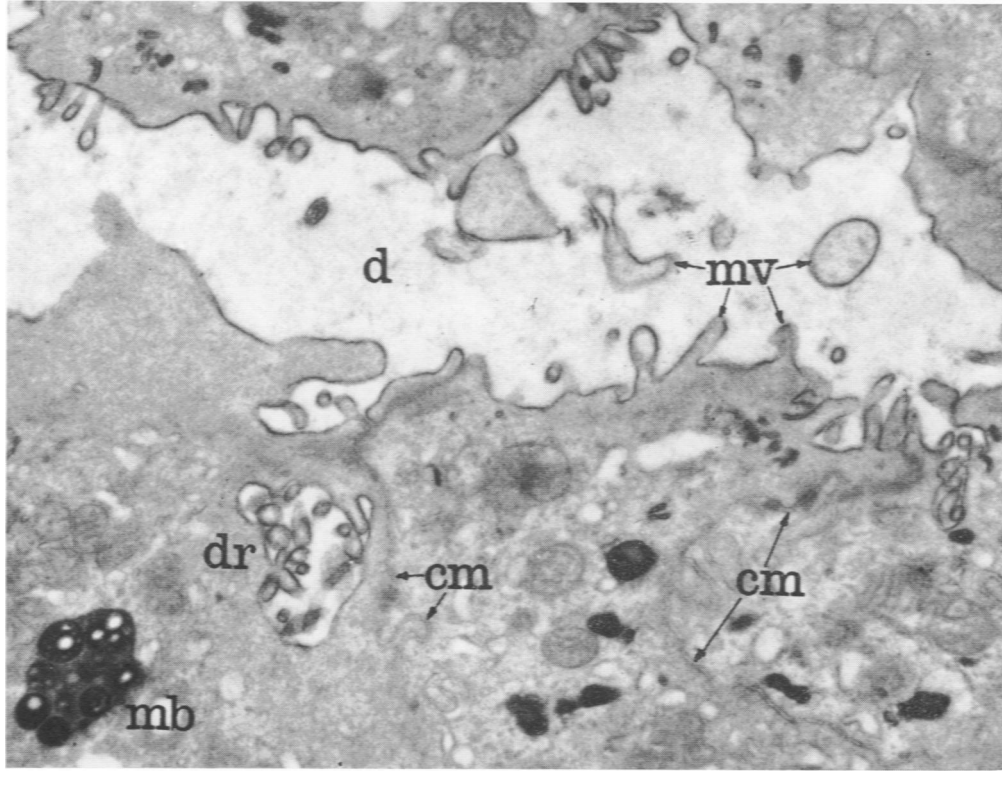

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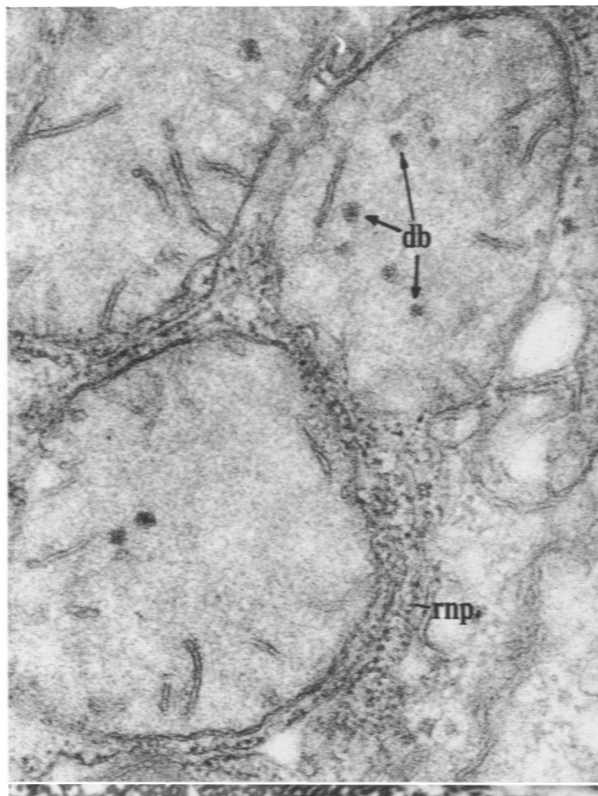
FIG. 14. Three mitochondria of a liver cell show the usual structure of the cristae mitochondriales which are short and appear to converge toward a hypothetical center of the corpuscle, leaving a large amount of matrix in the central portion. Note the fairly large number of "dense bodies" in the matrix. Lead hydroxide stain. × 52,800.

FIG. 15. A mitochondrion of a biliary epithelial cell shows the usual configuration of cristae traversing almost the entire lumen of the corpuscle, thereby dividing the matrix into numerous separate, yet communicating compartments. Note that this mitochondrion is considerably smaller than the average mitochondrion of liver cells. Phosphotungstic acid stain. × 83,600.

FIG. 16. A bile ductule is seen surrounded by intensely staining collagen fibrils in the surrounding connective tissue. The basement membrane which stains only faintly is seen here as a nonbranching structure. Note that the stain has also intensified the profiles of the lining of the microvilli. The lipid stains intensely as a result of its affinity for osmic acid. Phosphotungstic acid stain. × 20,720.



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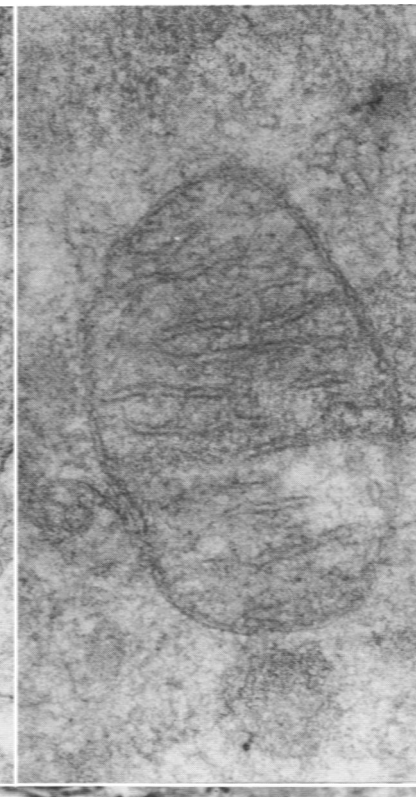

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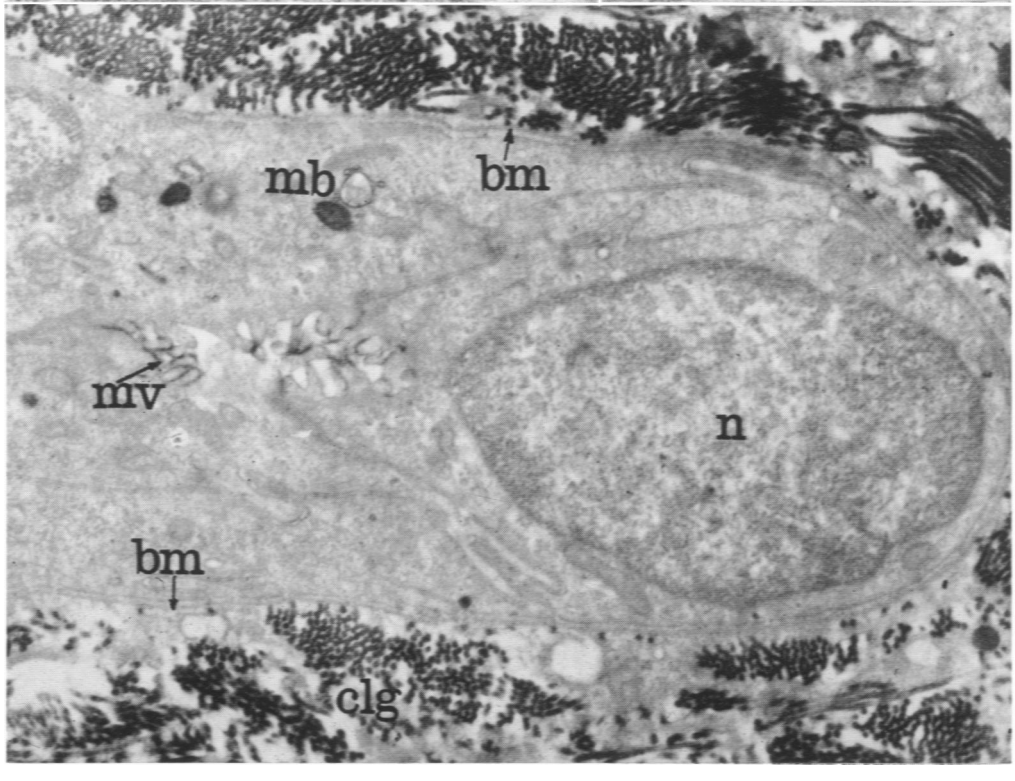

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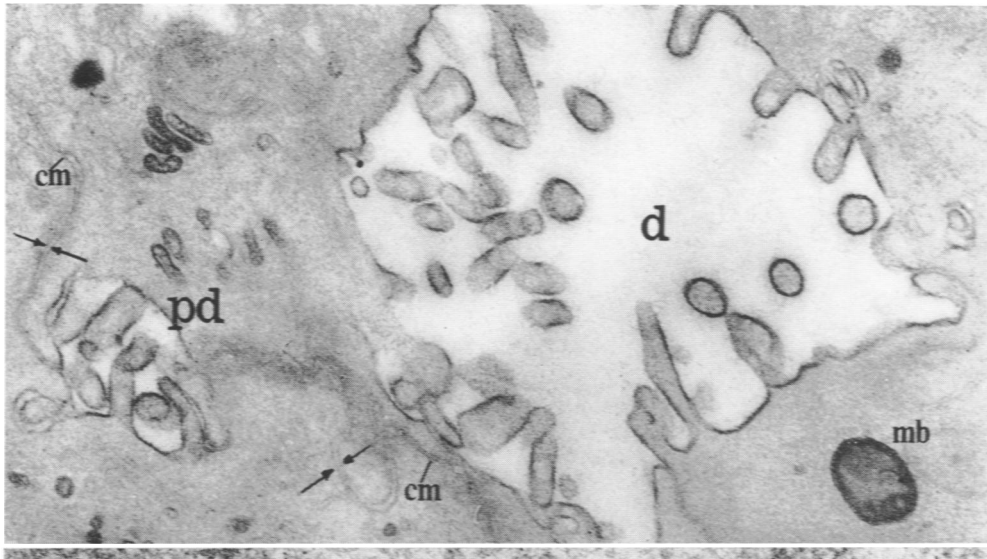
FIG. 17. The lumen of a bile ductule can be seen on the right of the photograph and a lumen of a duct of Hering is situated at the left margin. Note that the latter channel is formed by a separation of the cell membranes (arrows) of two adjacent biliary epithelial cells. Phosphotungstic acid stain. × 57,600.

FIG. 18. The interlocking plications of the cell membranes of two adjacent biliary epithelial cells can be seen. Note the nuclear membranes on either side of the cell boundaries. Lead hydroxide stain. × 68,800.

FIG. 19. The microvilli projecting into the lumen of a bile pre-ductule show the double-membraned lining accentuated by staining with this stain (arrow). Note that the staining of the adjacent cell membranes has not been accentuated. Phosphotungstic acid stain. × 84,800.



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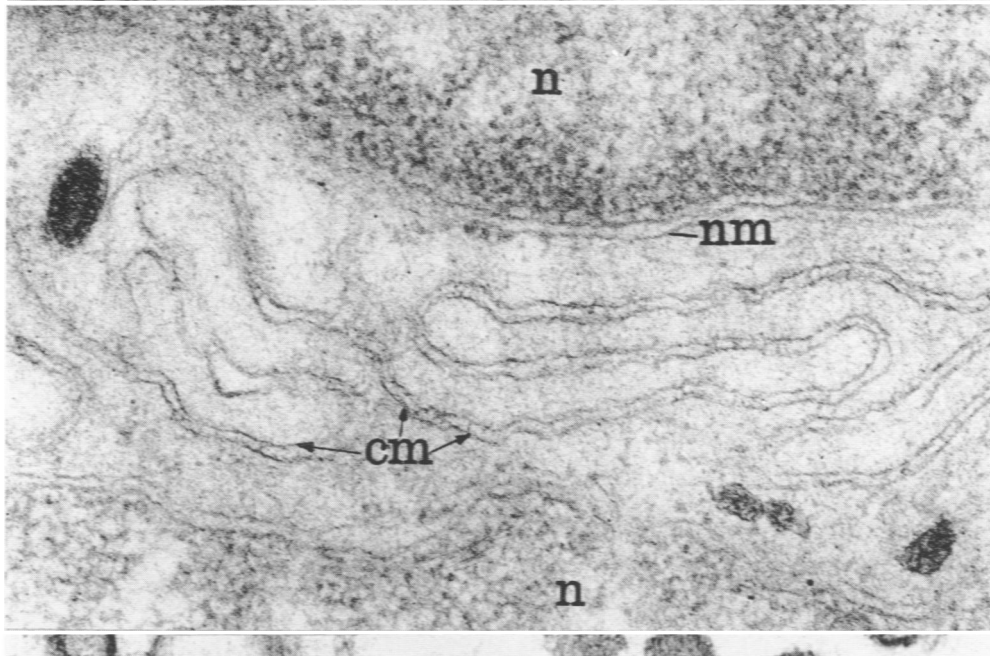

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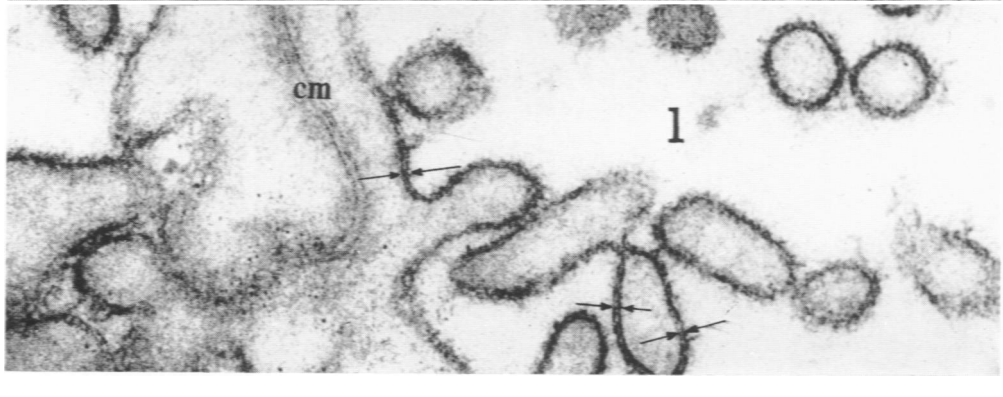

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